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The *Escherichia coli* Cell Division Protein ZipA Forms Homodimers Prior to Association with FtsZ

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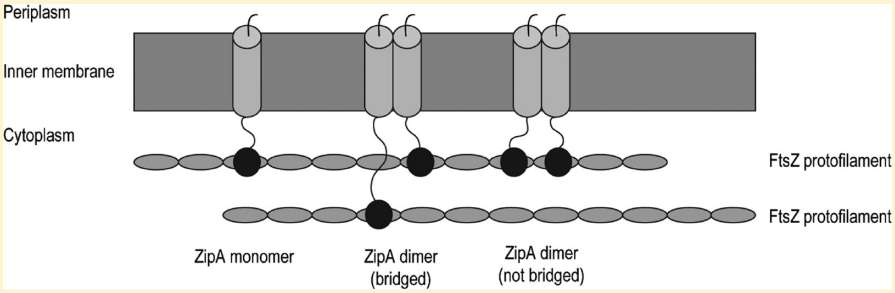

ABSTRACT: ZipA is an essential component of the cell division machinery in *E. coli* and other closely related bacteria. It is an integral membrane protein that binds to FtsZ, tethering it to the inner membrane. ZipA also induces bundling of FtsZ protofilaments and may play a role in regulating FtsA activity; however, the molecular details behind these observations are not clear. In this study we have analyzed the oligomeric state of ZipA *in vivo*, by chemical cross-linking, and *in vitro*, by native gel electrophoresis (BN-PAGE). Our data indicate that ZipA can self-associate as a homodimer and that this self-interaction is not dependent on the FtsZ-binding domain. This observation rules out the possibility that FtsZ polymers mediate the ZipA self-interaction. Given this observation, it is possible that a certain population of ZipA is recruited to the division septum in a homodimeric form.

Cell division in *Escherichia coli* is initiated by FtsZ, a structural homologue of tubulin,[1] which polymerizes into protofilaments at the division site (reviewed in refs 2−6). FtsZ subsequently recruits FtsA, ZipA, ZapA, ZapB, and ZapC, which facilitate bundling and anchoring to the inner membrane.[7−16] Together these proteins form a ringlike structure called the Z-ring. At least 20 other proteins are then recruited to the Z-ring, in a temporally coordinated process.[17−26] The assembly of these proteins into a multisubunit complex has been implied by colocalization experiments (reviewed in refs 21 and 26), pulldown assays,[27] and protein:protein interaction studies.[28,29]

A molecular understanding of the Z-ring has remained elusive. *In vivo* electron cryotomography images suggest that it is assembled from short overlapping protofilaments of FtsZ.[30] *In vitro* experiments are consistent with this observation, as they indicate that purified FtsZ can assemble into protofilaments in the presence of GTP.[31−33] These *in vitro* protofilaments subsequently form bundles in the presence of the early cell division proteins FtsA, ZipA, ZapA, ZapB, and ZapC.[7,9,12,14−16,34,35] These early cell division proteins are thus thought to be modulators of FtsZ assembly *in vivo*; however, their molecular mechanism is not clear.[3] Possible mechanisms have been suggested, which include (i) stabilizing FtsZ protofilaments, (ii) bridging two FtsZ protofilaments, and (iii) preventing depolymerization by inhibiting GTP hydrolysis.[2,7,8,11,12,34,36−38]

Since FtsA, ZapA, ZapB, and ZapC all self-interact,[7,11,14,28,29,34,36,39−41] it appears that homo-oligomerization is a general principle for proteins that modulate Z-ring assembly. However, the oligomeric state of ZipA is contentious. In a two-hybrid assay, the soluble cytoplasmic form self-interacts,[28] whereas it is monomeric when analyzed by static light scattering and analytical ultracentrifugation.[42] Studies of the oligomeric state of the full length and membrane-anchored form of ZipA have not been carried out, most probably because it is difficult to work with in solution. Moreover, it has a rare type I topology[43] and is incompatible with two-hybrid based protein:protein interaction methods for bacterial membrane proteins (i.e., TOXCAT,[44] GALLEX,[45] 434/P22,[46] T18/T25[29]), which require a type II topology. In this study we have probed the oligomeric state of ZipA *in vivo*, by chemical cross-linking, and *in vitro*, by native gel electrophoresis (BN-PAGE). Our data indicate that ZipA self-associates as a homodimer and that this self-interaction is independent of any association with FtsZ.

EXPERIMENTAL PROCEDURES

Strains and Plasmids. *ZipA* was amplified by PCR from the *E. coli* strain MG1655[47] using restriction endonuclease sites

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(5′*Xho*I/3′*Eco*RI) introduced into the oligonucleotide primers. The amplified fragment was digested and cloned into the *pGFPe* vector.[48,49] As the amplified *zipA* contained its native TGA codon, the encoded protein did not become fused to GFP. The QuickChange Site-Directed Mutagenesis Kit (Stratagene, Sweden) was used to insert TAA stop-codons, restriction sites, and cysteine residues. All constructs were confirmed by DNA sequencing (BM labbet AB, Sweden). The gene encoding 7L12A-GFP was generated by cloning a region encoding 7 leucines and 12 alanines[50,51] upstream of the gene encoding GFP in *pGFPe* using 5′*Nco*I/3′*Hind*III.

Strain CH45/pDB346[35,52] was grown in LB at 42 °C to an OD_{600} of 0.1, then switched to 30 °C for FtsZ depletion, and grown for a further 2.5 h. The control experiment (i.e., +FtsZ) was simply grown at 42 °C. Strain UT481/pDR144[53] was grown in LB media supplemented with 0.2% glucose at 37 °C to an OD_{600} of 0.3. SulA overexpression was induced by induction with 0.4 mM IPTG followed by growth for a further 2 h. The control experiment was simply not induced with IPTG. Strains CH45/pDB346 and UT481/pDR144 were gifts from Piet de Boer (Case Western Reserve University).

Protein Expression. Expression of ZipA was carried out as described previously,[54−56] but with a few minor modifications. Plasmids were transformed into either the *E. coli* strain SHuffle T7 Express *LysY* (New England Biolabs) or BL21(DE3)pLysS (Promega). A colony was inoculated into a 2 mL tube containing 1 mL of LB media with kanamycin (50 μg/mL) and incubated with vigorous shaking at 30 °C for 16 h. The culture was back-diluted 1/20 and incubated as before until the OD_{600} was between 0.3 and 0.5. Cells were harvested by centrifugation at 830*g* for 5 min and the cell pellet resuspended in 1 mL of M9 minimal media, supplemented with thiamine (10 mM) and all amino acids but methionine. The cells were then incubated for 90 min to starve them of methionine. Synthesis of the plasmid-encoded ZipA (which was under the control of the T7 RNA polymerase) was initiated by incubation of the cells with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 10 min (or 4 h for 7L12A-GFP). To suppress the production of native *E. coli* proteins, genomic transcription was suppressed by a further incubation with 0.2 mg/mL rifampicin for either 10 or 20 min (BL21(DE3)pLysS and SHuffle, respectively). Finally, radiolabeling was carried out by incubation with 15 mCi of ^{35}S-methionine for 10 min. The radiolabeling was carried out to enable detection of ZipA using autoradiography; however, when using the SHuffle strain, cleaner gels were obtained by probing with an antibody to ZipA, and these were therefore presented. Following the reaction, the cells were resuspended in 1 mL of LB media with kanamycin (50 mg/mL) and incubated for 30 min, to give the newly synthesized proteins a chance to self-assemble.

Cysteine Cross-Linking. Cysteine cross-linking was carried out as described previously.[54−56] A 50 μL aliquot of whole cells was collected from the labeling reaction, resuspended in 500 μL of PBS pH 7.5, and exposed to either 0.5 mM $CuPh_3$ (i.e., oxidizing conditions) or 50 mM DTT (i.e., reducing conditions). After 30 min on ice, the reaction was stopped by the addition of 10 mM *N*-ethylmaleimide, 20 mM EDTA pH 7.0. Cells were pelleted by centrifugation and resuspended in nonreducing SDS-PAGE loading buffer (i.e., without β-mercaptoethanol) and analyzed by SDS-PAGE. Proteins were detected by Western blotting using antibodies against ZipA (obtained from Piet de Boer, Case Western Reserve University) or GFP.

2D BN-/SDS-PAGE. BN-PAGE was carried out as described previously.[55,57] A useful review on the BN-PAGE method can be found in ref 58. To enable resolution in the size range of 60−100 kDa, proteins were separated on a 5−15% separating gel with dimensions of 14 cm × 20 cm × 1.5 mm, using the Hoefer SE-600 system.

Gel strips obtained from the BN-PAGE were soaked for 20 min in equilibration buffer [2% (w/v) SDS, 250 mM Tris-HCl pH 6.8] and then analyzed in a second dimension by SDS-PAGE. SDS-PAGE was performed in a Hoefer SE-600 system using a 4% stacking and an 8−16% separating gel (gel dimensions 14 cm × 20 cm × 1.5 mm). Gels were stained with Coomassie [10% (v/v) acetic acid, 45% (v/v) methanol, 0.25% (w/v) Coomassie R250] and protein spots detected using the PDQuest software (BioRad). Protein spots were excised from the gel and identified by Maldi-TOF and Maldi-TOF/TOF mass spectrometry (Alphalyse A/S, Denmark). Western blotting was carried out as described previously.[55] An antibody against FtsZ was obtained from William Margolin, University of Texas.

RESULTS

ZipA Can Be Cross-Linked As a Homodimer *in Vivo*. ZipA consists of four domains (Figure 1): The N-terminus contains a transmembrane domain that anchors the protein in the cytoplasmic membrane.[43] The cytoplasmic tail contains a charged domain, a domain enriched in proline and glutamine (P/Q), and an FtsZ-binding (FZB) domain. The charged and P/Q domains are thought to be unstructured,[43,59] thereby providing a flexible tether between the transmembrane domain and the FZB domain. To probe the oligomeric state of ZipA in the inner membrane of *E. coli* (i.e., *in vivo*), we initially used a cysteine cross-linking approach. Since ZipA does not contain

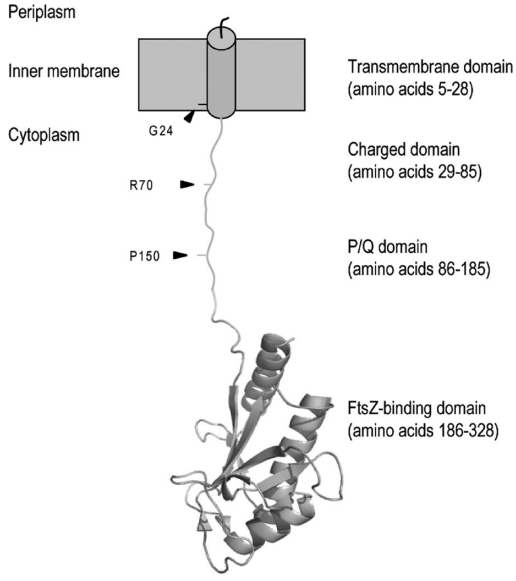

Figure 1. Domain architecture of ZipA. ZipA is anchored to the inner membrane of *E. coli* by a single transmembrane helix and has an N-out, C-in topology.[43] It consists of four major domains: a transmembrane domain, a charged domain, a domain enriched in proline and glutamine (P/Q), and an FtsZ-binding (FZB) domain. The structure of the FZB domain has been solved (PDB: 1F47), but all other domains are drawn in cartoon format. Amino acids that were mutated to cysteine in this study are annotated.

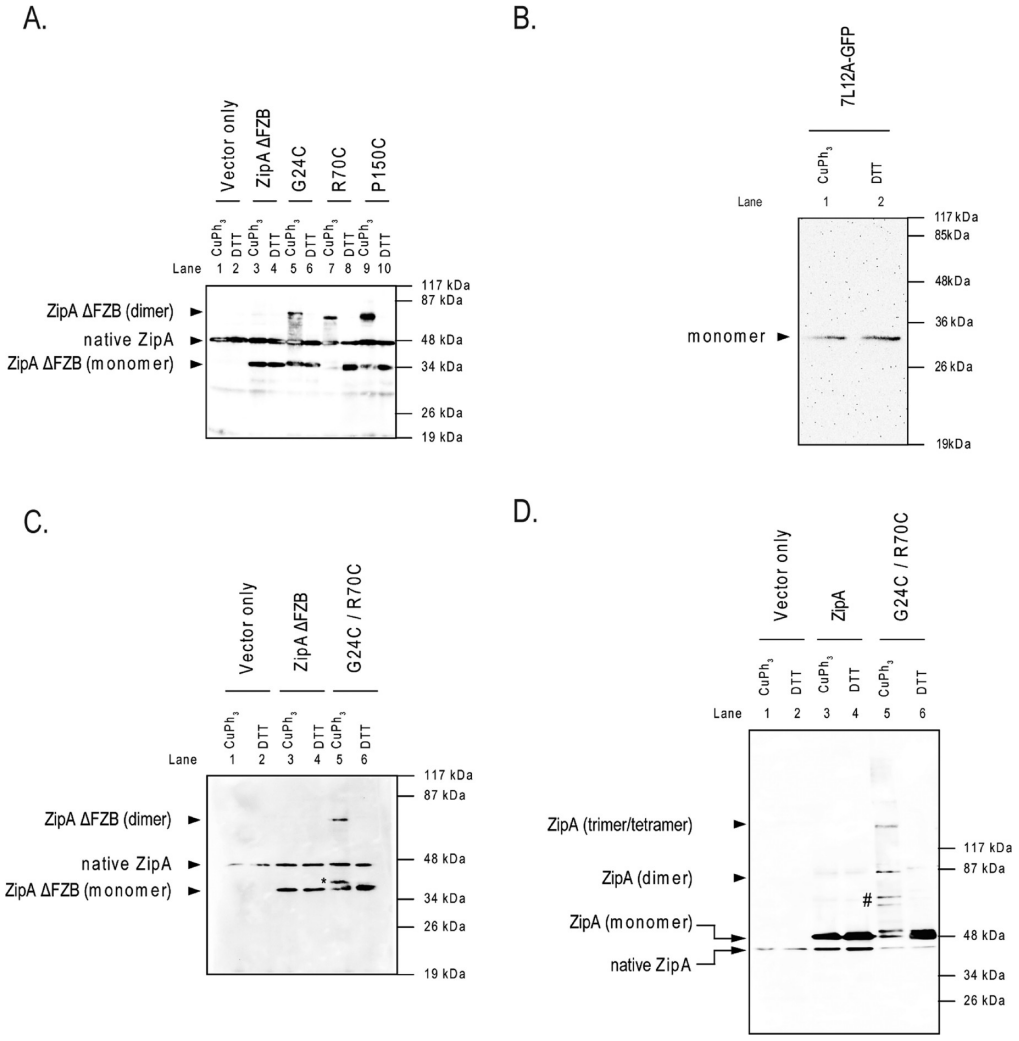

Figure 2. ZipA can be cross-linked *in vivo*. Whole cells containing single-cysteine mutants of ZipA were exposed to oxidizing conditions ($CuPh_3$) or reducing conditions (DTT) prior to analysis by nonreducing SDS-PAGE. The gels were probed with either a ZipA antibody or a GFP antibody. (A) ZipA lacking the FtsZ-binding domain (ZipA ΔFZB), (B) the artificial membrane protein 7L12A-GFP, (C) a double-cysteine mutant of ZipA lacking the FtsZ-binding domain (ZipA ΔFZB), or (D) a double-cysteine mutant of full-length ZipA. Cross-linked products and monomers and native ZipA are indicated. Note that the native ZipA (i.e., chromosomally encoded) migrates slightly faster in the SDS-PAGE than the cysteine mutants (i.e., plasmid encoded). For all gels the molecular mass marker is indicated.

native cysteines, we substituted them into positions G24, R70, or P150 (Figure 1). In this initial experiment the FZB domain was omitted, as we believed that binding to FtsZ protofilaments could bring ZipA molecules into close proximity, thus creating secondary polymers (i.e., polymers mediated by FtsZ). The single-cysteine mutants were expressed in a strain of *E. coli* that enables disulfide bonds to form in the cytoplasm and whole cells were exposed to either oxidizing (i.e., 0.5 mM $CuPh_3$) or reducing conditions (i.e., 50 mM DTT). When the cells that had been exposed to oxidizing conditions were analyzed by nonreducing SDS-PAGE and probed with an antibody to ZipA, we could detect a gel shift that was at the expected size of a ZipA ΔFZB homodimer (Figure 2A, lanes 5, 7, and 9). On average, ≥40% of the protein was shifted to the homodimeric form. As expected, the gel shift was not detected when the same cells were exposed to reducing conditions (Figure 2A, lanes 6, 8, and 10). Moreover, we could not detect gel shifts of the native ZipA or ZipA ΔFZB (both of which are cysteine-less) (Figure 2A, lanes 1 and 3). To ensure that the cysteine cross-linking approach was not generating false positive cross-links, we also expressed an artificial membrane protein constructed from a transmembrane helix containing 7 leucines and 12 alanines[50,51] fused to a monomeric version of GFP. 7L12A-GFP contains two cysteine residues, one of which is surface exposed and which could potentially form a cysteine cross-link if our methodology artificially generated false positive cross-links. When expressed in *E. coli* 7L12A-GFP associated with the inner membrane (data not shown), but could not be cross-linked under oxidizing conditions (Figure 2B). This control experiment indicates that the cysteine cross-links do not occur spontaneously and that the approach is appropriate for analyzing the oligomeric state of ZipA. Taken together, these data indicate that ZipA ΔFZB G24C, R70C, and P150C can all be detected as homodimers *in vivo* in *E. coli*.

Cross-linking experiments using proteins with single-cysteine residues can only report on homodimers, not higher oligomers. To determine if ZipA ΔFZB formed higher oligomers, we also created a double-cysteine mutant G24C/R70C. When expressed in *E. coli* and analyzed in the same way as the single-cysteine mutants, we again detected a gel shift that was

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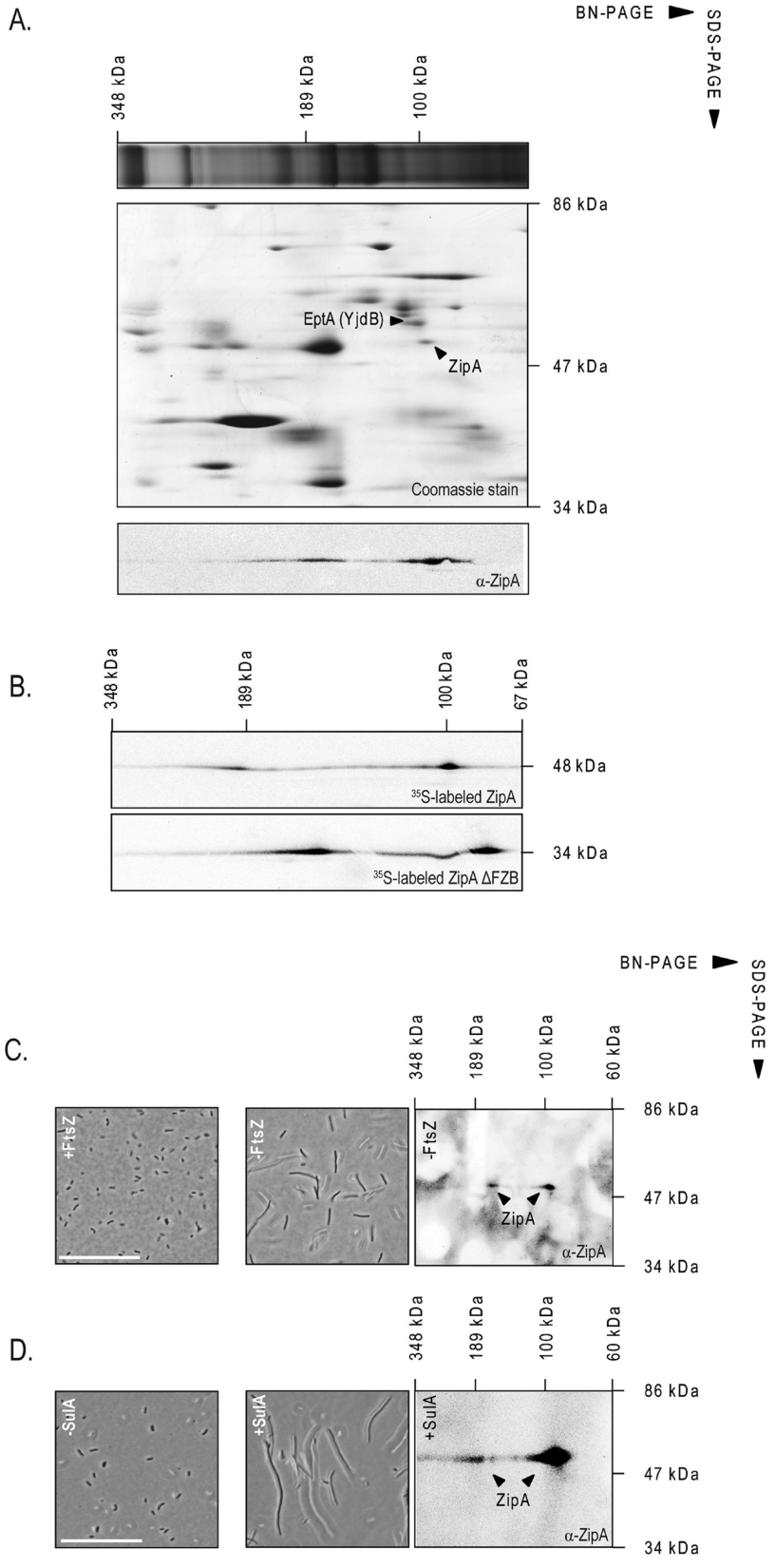

Figure 3. Native ZipA can be detected as two forms by 2D BN-/SDS-PAGE. (A) *E. coli* membrane protein complexes were solubilized from inner membrane vesicles, then separated by nondenaturing BN-PAGE (top strip) and a second dimension SDS-PAGE and either stained with Coomassie (middle panel), or blotted onto a nitrocellulose membrane and probed with an antibody against ZipA (lower panel). Protein spots indicated with an arrowhead were identified by mass spectrometry (ref 55 and this study). (B) Plasmid-encoded ZipA and ZipA ΔFZB were labeled with ^{35}S-methionine using the rifampicin-blocking technique, then separated by 2D BN-/SDS-PAGE, and detected by autoradiography. (C) Analysis of native ZipA when FtsZ is depleted. Cell phenotypes are shown in the microscope images to the left, and a Western blot of inner membrane complexes

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Figure 3. continued

separated by BN-/SDS-PAGE and probed with ZipA antibody is shown to the right. Scale bars represent 50 μm. (D) Analysis of native ZipA in the presence of SulA, an inhibitor of FtsZ polymerization. See above for annotation. Molecular mass markers are indicated.

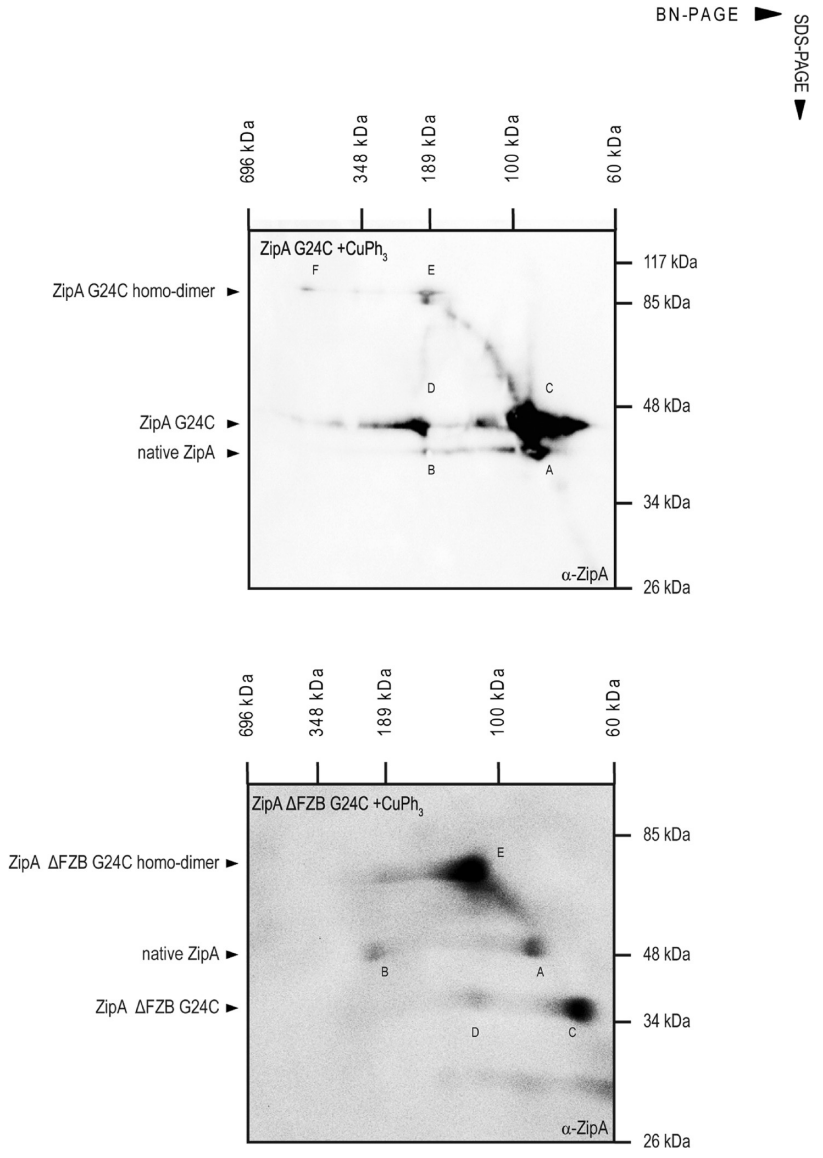

Figure 4. The two forms of ZipA in the 2D BN-/SDS-PAGE correspond to monomers and homodimers. Whole cells containing single-cysteine mutants of ZipA and ZipA ΔFZB (called ZipA G24C and ZipA ΔFZB G24C, respectively) were exposed to oxidizing conditions ($CuPh_3$), then separated by BN-/SDS-PAGE, and probed with an antibody to ZipA. Only the higher molecular mass forms of ZipA G24C and ZipA ΔFZB G24C that were detected in BN-PAGE (spots labeled D) could be cross-linked and detected as homodimers in the SDS-PAGE (spots labeled E). The lower molecular mass forms in the BN-PAGE (spots labeled C) were unable to be detected as homodimers in the SDS-PAGE, thus indicating that they are monomers. The native forms of ZipA (i.e., chromosomally encoded) are marked with A and B. The single-cysteine mutants (i.e., plasmid encoded) are marked with C–F. Molecular mass markers are indicated.

consistent with the formation of a homodimer (Figure 2C, lane 5). We also detected a lower molecular mass cross-link that has either arisen through intramolecular cross-linking of G24C and R70C or from a interaction with another cysteine containing protein (marked with an asterisk in Figure 2C, lane 5). We conclude that ZipA ΔFZB G24C/R70C can be trapped as a homodimer but not a higher homo-oligomer. Since the FZB domain of ZipA is required for recruitment to the division site and association with FtsZ,[8] the ZipA ΔFZB homodimer must form prior to localization at the septum and independently of any interaction with FtsZ.

ZipA Forms Higher Oligomers in the Presence of FtsZ.

To determine if the full length ZipA had the same oligomeric state as ZipA ΔFZB, we repeated the cysteine cross-linking with the full length ZipA protein. Again we could detect a gel shift corresponding to a cross-linked homodimer when cells expressing ZipA G24C, R70C, or P150C were subjected to oxidizing conditions (data not shown). However, when cells expressing the double-cysteine mutant ZipA G24C/R70C were

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subjected to oxidizing conditions, we could detect additional gel shifts (Figure 2D, lane 5, vs Figure 2C, lane 5). On average, ≥20% of the protein was shifted to each of these higher oligomeric forms. Most notably we could detect a gel shift corresponding to the molecular weight of a ZipA trimer/tetramer. The simplest explanation for this gel shift is that three or four copies of ZipA G24C/R70C are brought into close proximity through binding to FtsZ, thus enabling them to be cross-linked by oxidation of the cysteine residues. We could also detect two lower molecular gel shifts that have either arisen through intramolecular cross-linking of G24C and R70C or cross-linking with other cysteine containing proteins in the cytoplasm (marked with # in Figure 2D, lane 5). Note that FtsZ can be ruled out as an interaction partner as it does not contain any cysteine residues. Further work will be required to resolve the identity of these cross-links.

Analysis of Native ZipA by BN-PAGE. In a parallel approach we probed the oligomeric state of the native (i.e., chromosomally encoded) ZipA by blue native polyacrylamide gel electrophoresis (BN-PAGE), a widely used electrophoretic method for separating membrane proteins (reviewed in ref 58). In this experiment inner membrane vesicles were purified from wild-type *E. coli* cells, the entire inner membrane proteome was solubilized with n-dodecyl β-D-maltoside, and the detergent-protein complexes were analyzed by nondenaturing BN-PAGE (Figure 3A, top panel). To facilitate the identification of proteins, the BN-PAGE was coupled to a second dimension of denaturing SDS-PAGE (Figure 3A, middle panel). Mass spectrometry analysis of Coomassie stained protein spots in the 2D BN-/SDS-PAGE enabled identification of ZipA (ref 55 and this study) in a vertical channel that was devoid of other protein spots. This observation indicates that ZipA had migrated independently in the BN-PAGE and had no heterointeraction partners. While we had previously noted that the ZipA resolved in a vertical channel with the phosphoethanolamine transferase EptA (formerly YjdB),[55] it was not the case in this study (where we have used longer gels), and we conclude that there is no physical interaction between ZipA and EptA.

Intriguingly, a second form of ZipA could be detected when the 2D BN-/SDS-PAGE was probed with an antibody to ZipA (Figure 3A, lower panel). This form corresponded to ∼30% of the detected ZipA but most likely escaped detection by mass spectrometry because there were other abundant proteins in the same area of the 2D BN-/SDS-PAGE. The same two forms were detected when plasmid-encoded versions of ZipA and ZipA ΔFZB were separated by 2D BN-/SDS-PAGE (Figure 3B). This observation indicates that the plasmid-encoded versions of ZipA and ZipA ΔFZB behave in the same way as the native ZipA when separated by 2D BN-/SDS-PAGE.

To determine if either of the native ZipA forms was dependent on FtsZ, we reanalyzed native ZipA in conditions where FtsZ had been depleted. In this experiment an *ftsZ* null strain (CH45) was supplemented with a plasmid containing *ftsZ* under the control of a *λR* promoter and a temperature-sensitive allele of a *λ* repressor (pDB346).[35] When the strain was grown at 42 °C (i.e., +FtsZ), the cells formed small rods and mini cells, but when cells were grown at 30 °C (i.e., −FtsZ), they formed filaments, indicating that FtsZ had been depleted (Figure 3C). Quantitative immunoblot analysis indicated that the level of FtsZ in the depleted cells was ∼13% of the levels in the control cells (data not shown). Nevertheless, we could detect both forms of ZipA when inner membrane vesicles were isolated from the −FtsZ cells and analyzed by 2D BN/SDS-PAGE (Figure 3C), indicating that both forms of ZipA in the 2D BN/SDS-PAGE could form even when the levels of FtsZ were too low to support cell division.

We also tested whether both forms of the native ZipA could be detected in conditions where FtsZ was prevented from polymerizing at the midcell by the expression of SulA (SfiA).[60,61] In this experiment the UT481 strain was transformed with the plasmid-borne *sfiA* gene under the control of a *lac*-promoter (pDR144). Following induction of SulA expression with IPTG the cells became filamentous, indicating that SulA had prevented FtsZ polymerization (Figure 3D). BN-PAGE and Western blotting of inner membrane complexes indicated that ZipA was present as two forms, in the presence of SulA.

To determine if either of the two native ZipA forms found in the 2D BN-/SDS-PAGE corresponded to the homodimer we had previously noted, we separated the oxidized ZipA G24C and ZipA ΔFZB G24C by 2D BN-/SDS-PAGE and probed with an antibody to ZipA. Our rationale was that a homodimeric form should give a predictable gel shift in the second dimension SDS-PAGE, whereas a monomer should not. Analysis of the blots enabled identification of ZipA in multiple places in the gel (labeled A−F, Figure 4). Spots corresponding to the native forms of ZipA that we have described earlier were labeled A and B. These spots were less abundant than the spots corresponding to the plasmid-encoded forms of ZipA, which are labeled C−F. In these experiments the spots corresponding to the higher molecular mass forms of ZipA G24C and ZipA ΔFZB G24C in BN-PAGE (labeled D) were shifted in the SDS-PAGE (see spots labeled E), thus indicating that they are homodimers. In contrast, the spots corresponding to the lower molecular mass forms in the BN-PAGE (labeled C) were not shifted in the SDS-PAGE. Taken together with our earlier observation that this form was migrating independently in the BN-PAGE (see above), we conclude that spot C is a monomeric form of ZipA. A sixth form of ZipA could also be detected in the experiment with ZipA G24C (labeled F). The origin of this form is not currently known and is under investigation.

The BN-PAGE analysis indicates that native ZipA is distributed between monomeric and homodimeric populations when detergent solubilized. Efforts to further substantiate the stoichiometries of the two forms using molecular weight standards were not successful as ZipA migrates unpredictably during gel electrophoresis. For example, the monomeric form of ZipA is predicted to have a molecular mass of 36 kDa yet migrates at ∼50 kDa in the SDS-PAGE[43] (and this study) and ∼90 kDa in the BN-PAGE (this study).

DISCUSSION

Current models of cell division do not provide a clear mechanism for how early cell division proteins (i.e., FtsA, ZipA, ZapA, ZapB, and ZapC) facilitate polymerization or induce lateral associations between FtsZ molecules and bring about bundling of FtsZ protofilaments. For ZipA it has been suggested that an interaction between the FZB domains of two ZipA molecules could provide a noncovalent cross-link (or bridge) for FtsZ protofilaments.[8] However, recent data indicate that the cytoplasmic domains of ZipA are monomeric in solution,[42] thus ruling out the possibility that an FZB−FZB interaction could occur. In this study we have analyzed the oligomeric state of the full length, membrane anchored ZipA

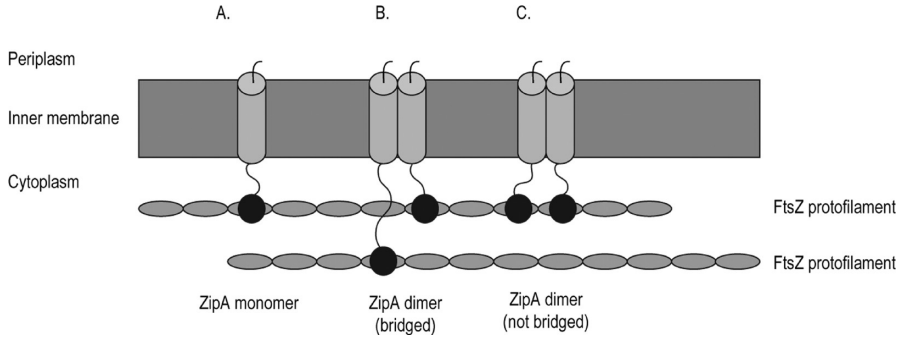

Figure 5. A putative mechanism for how ZipA homodimers could mediate Z-ring assembly. (A) Monomeric ZipA can mediate membrane attachment, but it is not clear how it could mediate bundling of FtsZ protofilaments. (B) Homodimeric ZipA could bind two FtsZ protofilaments by a bridging mechanism. This would enable bundling of FtsZ protofilaments. (C) Homodimeric ZipA could bind to a single FtsZ protofilament, thereby facilitating polymerization and/or stabilization of FtsZ protofilaments.

using an *in vivo* cysteine cross-linking approach. In our initial set of cross-linking experiments, the FtsZ-binding domain (FZB) of ZipA was omitted, as we believed that binding to FtsZ protofilaments could create secondary polymers (i.e., polymers mediated by FtsZ). Our data indicate that ZipA ΔFZB can be detected as a homodimer when cysteine residues are substituted into positions G24, R70, or P150. We also tested whether we could detect higher homo-oligomers, by substituting double cysteines at G24/R70, but we could not. When we repeated the cysteine cross-linking approach on a double-cysteine mutant G24/R70 that contained the FtsZ-binding domain, we could detect a higher oligomeric form of ZipA that may be either a homotrimer or a homotetramer. These data clearly indicate that ZipA ΔFZB is able to form a homodimer, prior to its association with FtsZ. Moreover, our data indicate that interactions with FtsZ bring ZipA molecules together so that they can be cross-linked into a higher oligomeric form.

To substantiate the observation that ZipA could form a homodimer in the inner membrane of *E. coli*, we analyzed the native ZipA by 2D BN-/SDS-PAGE. This approach indicated that ZipA migrated in two forms. The composition of these two forms was difficult to deconvolute from the 2D BN-/SDS-PAGE. To resolve the identity of the two forms, we analyzed the oxidized ZipA G24C and ZipA ΔFZB G24C by 2D BN-/SDS-PAGE. In these experiments the cross-linked homodimers migrated in the same way as the higher molecular mass form of the native protein in the BN-PAGE. This experiment indicated that the higher molecular mass form of the native ZipA that we had previously observed in the BN-PAGE was in fact a homodimer. Moreover, and in agreement with our earlier observations, the homodimeric form could still be detected when FtsZ was depleted from the cells and when FtsZ was prevented from polymerizing. The data presented therefore indicate that there are two populations of ZipA in the inner membrane of *E. coli*: a monomeric population and a homodimeric population.

So what is the biological significance of a ZipA homodimer? Since ZipA homodimers were detected when the FZB domain was omitted, we reason that they must have formed prior to association with FtsZ at the midcell. We therefore speculate that ZipA is recruited to the division septum in two forms: a monomeric form and a homodimeric form. Upon arrival at the septum the homodimeric form could bind to a single FtsZ protofilament, thus facilitating polymerization or stabilization of polymers. The homodimeric form could also "bridge" adjacent FtsZ protofilaments, thus facilitating bundling (Figure 5). Further work is required to determine if these forms of ZipA contribute differently to cell division.

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ABBREVIATIONS

BN-, blue native-; FZB, FtsZ-binding domain; $CuPh_3$, copper phenanthroline; DTT, dithiothreitol; DDM, *n*-dodecyl β-D-maltoside.

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